



Transfection with NEU1-encoding plasmid increases NEU1 activity in HEK293T cells. HEK293T cells seeded at a density of 2×10^6 cells per 10 cm dish were untransfected or transiently transfected with 6 μ g of bicistronic plasmid CathA-IRES-NEU1 for 48 h using lipofectamine. Sialidase activity in cell homogenates was measured against 0.2 mM 4MU-NeuAc [2'-(4-methylumbelliferyl)- α -D-N-acetylneuraminic acid] in Na-acetate buffer, pH 4.6 for 30 min at 37°C. The reaction was terminated by the addition of 0.4 M glycine buffer, pH 10.4. The fluorescence of liberated 4-methylumbelliferone was measured using a Shimadzu RF-5301 spectrofluorometer with excitation at 355 nm and emission at 460 nm and used to calculate specific activity. Protein concentration was measured according to the Bradford method. Data are shown as means \pm s.e.m of three independent experiments. *** Statistically significant difference as measured by *t*-test ($P < 0.001$).